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Reassessing The Role Of TCF4 Variants In Fuchs Endothelial Corneal Dystrophy

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Abstract

Background/Objectives:

Fuchs endothelial corneal dystrophy (FECD) is a genetically heterogeneous disorder in which *TCF4* trinucleotide repeat (TNR) expansion is the strongest known genetic risk factor. Several non-coding *TCF4* single nucleotide polymorphisms (SNPs) have also been associated with FECD, although their contribution remains unclear. We investigated their relationship with disease susceptibility and severity.

Subjects/Methods:

The primary cohort comprised 61 unrelated FECD patients from Spanish families and 23 unaffected relatives. Additional relatives from 11 families were recruited for familial analyses. *TCF4* SNPs and TNR expansion were analysed. Associations with FECD, SNPs and TNR expansion co-occurrence, predictive performance for TNR expansion, familial co-segregation, and clinical characteristics were evaluated.

Results

The rs613872 and rs784257 risk alleles and expanded *TCF4* alleles were more frequent in affected individuals. Co-occurrence between SNPs and TNR expansion consistent with linkage disequilibrium was observed suggesting that SNPs are commonly co-inherited with expanded alleles rather than acting as independent risk factors. Rs613872 showed strong predictive performance for TNR expansion. Family analyses showed no co-segregation and incomplete penetrance between TNR expansion and FECD in some families. Within-family comparisons suggested that expansion carriers may have more severe disease, including poorer visual acuity, greater corneal thickness, and higher likelihood of corneal transplantation.

Conclusions

TCF4 TNR expansion is strongly associated with FECD but does not segregate with the disease and shows incomplete penetrance in some families. Rs613872 may serve as an accessible marker for TNR expansion. Familial analyses suggest that the expansion may contribute to disease severity, although validation in larger cohorts is required.

Plain English summary

Fuchs endothelial corneal dystrophy (FECD) is a common inherited eye disease that affects the inner layer of the cornea and can gradually lead to blurred vision and, in severe cases, the need for corneal transplantation. Changes in a gene called *TCF4* are known to play an important role in the disease, particularly an expansion of a repeated DNA sequence.

In this study, we analysed *TCF4* genetic changes in Spanish families affected by FECD. We found that some commonly studied genetic variants were frequently inherited together with the *TCF4* repeat expansion, suggesting that these variants may act mainly as markers of the expansion rather than as independent causes of disease. One variant, rs613872, was particularly useful for predicting whether a person carried the expansion.

Our family-based analyses also showed that carrying the *TCF4* expansion does not always lead to FECD, indicating that other genetic or environmental factors may influence whether the disease develops. In addition, carriers of the expansion tended to show more severe clinical features.

These findings improve our understanding of how TCF4 contributes to FECD and may help refine genetic assessment and future studies of disease risk and severity.

Introduction

Corneal dystrophies comprise a heterogeneous group of inherited disorders that can lead to significant visual impairment and, in advanced cases, blindness. Among them, Fuchs endothelial corneal dystrophy (FECD) affects approximately 4–7% of the adult population worldwide and is the leading cause of corneal transplantation. It is more common in women than men with a female-to-male ratio of 2–3:1(1–7).

FECD is a bilateral, often asymmetric disorder characterised by progressive loss of corneal endothelial cells (CEC) and accumulation of abnormal extracellular matrix deposits, known as guttae, in Descemet membrane (DM). These changes impair endothelial function, leading to altered fluid regulation and progressive corneal oedema. Patients are typically diagnosed in the fifth to sixth decade of life and may present with blurred vision, glare, and, in advanced stages, substantial visual loss and ocular pain. Clinically, FECD is associated with reduced endothelial cell density, pleomorphism, polymegathism, increased central corneal thickness (CCT), and, in advanced disease, epithelial or subepithelial bullae. A possible association with ocular hypertension or glaucoma has also been proposed, although this remains controversial(8–17).

Corneal transplantation remains the conventional treatment for advanced FECD but is limited by donor tissue availability and surgery-related risks. Consequently, pharmacological, cell-based and gene-based approaches are under investigation.

Rare pathogenic variants in *COL8A2*(18–21), *SLC4A11*(22–25) and *ZEB1*(26–29) genes, which are involved in extracellular matrix regulation, endothelial cell survival and ion transport, have been described but account for only a small proportion of familial FECD cases. The strongest and best-characterised genetic risk factor is an intronic CTG trinucleotide repeat (TNR) expansion in *TCF4*. *TCF4* encodes a transcription factor involved in neurodevelopment and gene regulation, and variants affecting its function have also been associated with disorders such as Pitt-Hopkins syndrome and schizophrenia.

The combination of short tandem repeat (STR) analysis and triplet repeat-primed PCR (TP-PCR) is widely used to determine *TCF4* TNR length in leukocyte DNA. The association between expanded alleles, usually defined as > 50 repeats, and FECD has been replicated across multiple cohorts(1–5, 30–38). Although expanded alleles can also be detected in a small proportion of unaffected individuals, they are strongly associated with FECD. Proposed pathogenic mechanisms include disruption of *TCF4* isoform expression, toxic RNA foci, sequestration of RNA-binding proteins, widespread missplicing, repeat-associated non-AUG translation, and somatic instability. However, the relationship between repeat length and clinical severity remains uncertain(2, 31, 33, 39–48).

In addition to the *TCF4* TNR expansion, several single nucleotide polymorphism (SNP) variants located in the upstream and non-coding regions of *TCF4* have also been reported to be more frequent in individuals with FECD than in healthy populations. Among them, rs613872 (T > G) is the most extensively studied and consistently associated with disease risk. Other variants, including rs11659764 (T > A), rs784257 (C > T), rs17089887 (T > C), and rs17595731 (G > C), have also been linked to FECD susceptibility (1, 2, 30, 32, 35, 37, 49–54).

This study aims to evaluate the distribution of the risk alleles for these FECD associated SNPs, as well as *TCF4* TNR expansion length, in a Spanish cohort of FECD patients, and to assess their contribution to disease susceptibility and clinical severity.

Materials and methods

Participants recruitment and sample collection

Individuals from 61 families were recruited across nine Spanish ophthalmology clinics belonging to the Miranza Group (Miranza Inversiones Oftalmológicas, S.L.). The initial cohort included 61 primary cases diagnosed with FECD, defined by reduced endothelial cell count (ECC) and the presence of corneal *guttae*, as well as 23 unaffected relatives aged over 40 years. To enable familial co-segregation analyses, additional 14 affected relatives and one further unaffected relative over 40 years of age were subsequently included. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of IMO (protocol code COD IMO 240716-271, date of approval 07/23/2024). Written informed consent was obtained from each individual. Genomic DNA was extracted from peripheral blood or saliva using the Mag-Bind Blood DNA HDQ system (Omega Bio-Tek, Norcross, GA, USA).

Clinical evaluation

All participants underwent a comprehensive ophthalmic examination including visual acuity, refraction, intraocular pressure, axial length, and anterior chamber depth using optical coherence tomography (OCT). Slit-lamp examination assessed *guttae* and other corneal changes, while specular microscopy evaluated endothelial cell density, pleomorphism, and polymegathism. Central corneal thickness (CCT)

was measured by pachymetry, and Pentacam imaging was used, where available, to assess corneal densitometry, the Index of Vertical Asymmetry (IVA), and the Belin–Ambrosio Enhanced Ectasia Display (BAD) total deviation values. For transplanted eyes, preoperative clinical data were collected when available.

TCF4 expansion genotyping by STR and TP-PCR assay

TCF4 TNR length was determined by Eurofins/Megalab Laboratory (Barcelona, Spain). A combination of PCR-based methods short tandem repeat (STR) and triplet repeat primed polymerase chain reaction assay (TP-PCR) was carried out to analyse the 61 primary FECD patients and the 23 non-affected relatives.

PCR amplification and Sanger sequencing

PCR amplification of the desired upstream and intronic FECD-associated SNPs in *TCF4* was performed for the 61 primary FECD patients of each family and 23 unaffected relatives older than 40 years old. The PCR products were purified using 96-well Acroprep Advance plates (Pall Corporation, Ann Arbor, MI, USA) with a vacuum manifold (Pall Corporation). All primer sequences used in this study are listed in Table S1. The products were Sanger sequenced using the reverse primer in all cases (Table S1.) Sanger sequencing results were downloaded from the manufacturer's platform and the data were aligned and analysed.

Familial studies

In order to evaluate co-segregation of the *TCF4* TNR expansion and FECD, additional relatives from 11 families were recruited to ensure a minimum of three individuals per family, with at least two affected members in each pedigree. In total, 14 relatives diagnosed with FECD and one unaffected relative were included to perform this co-segregation analysis.

The rs613872 variant was determined in the initial cohort of 61 primary FECD cases and 23 unaffected relatives as a potential predictor of the presence of the expanded *TCF4* allele. Subsequently, this finding was evaluated in the familial co-segregation cohort to assess the consistency and robustness of its predictive performance. Both genetic markers were analysed as described in the Materials and Methods section.

Statistical analysis

Statistical difference in allele distribution for each *TCF4* SNP and TNR expansion between groups were assessed using Fisher's exact test, whereas difference in genotype distributions were evaluated using the chi-square (χ^2) test. Significant differences in clinical parameters between groups were analised

using an unpaired T-test and are presented as mean ± SEM. Statistical analysis was performed with GraphPad Prism 8.0.1 program.

Results

Description of the FECD cohort.

The cohort included 61 unrelated FECD patients and 23 unaffected relatives older than 40 years. In the FECD group the mean age was 62.97 years (range 32–89) and for the healthy control group 63.00 years (range 51–77). Females were more frequent among FECD patients (80.33% vs 65.22%) and a higher proportion of individuals in the FECD group presented with cataracts or had previously undergone phacoemulsification with intraocular lens implantation (52.46% vs 26.09% in healthy relatives). Corneal transplantation had been performed in 17 affected individuals (27.87%), predominantly by DMEK, with four cases of penetrating keratoplasty.

Association between risk allele of rs613872 and rs784257 with FECD in Spanish cohort.

Several SNPs in *TCF4* previously associated with FECD in the literature were analysed in our cohort of 61 primary FECD patients and 23 unaffected relatives. Among them, only rs613872, the most extensively studied variant, and rs784257 showed a significant association with FECD compared with the non-affected control group. The G allele of rs613872 was identified as the risk allele (40.98% alleles in FECD vs 15.22% in non-affected relatives) and the T allele of rs784257 was also associated with the presence of FECD (36.07% alleles in FECD vs 15.22% in healthy relatives) (Table 1).

Both risk variants were predominantly observed in FECD cases in the heterozygous state, accounting for more than half of the FECD cohort (59.02% subjects for rs613872 and 62.29% for rs784257). Homozygosity for the risk allele was less frequent, observed in 11.47% FECD individuals of rs613872 and 4.92% for rs784257 (Table 1).

Moreover, we observed co-occurrence of SNPs rs613872 and rs784257 within the same individuals in both the non-affected and FECD groups. In both cohorts, individuals showed a tendency to carry either both risk alleles (30.44% subjects in healthy relatives and 65.57% in FECD) or neither (69.57% individuals in healthy controls and 27.87% in FECD) (Fig. 1A). Only the FECD group included patients carrying a single risk variant, with 4.92% subjects presenting rs613872 alone and 1.64% rs784257 alone.

Additional *TCF4* SNPs also described in the literature associated with FECD rs11659764, rs17089887 and rs17595731 were evaluated but did not show a significant association with the disorder (Table 1).

Distribution of TCF4 TNR length in Spanish cohort.

TCF4 TNR length was evaluated in our cohort using a threshold of ≥ 50 repeats to define expanded alleles associated with FECD susceptibility. No alleles with 41–49 repeats were identified.

The allele frequency distribution in both healthy control and FECD groups showed that expanded alleles were more prevalent in the FECD cohort (35.25% alleles), although they were also present in the non-affected group (6.52% alleles). In the non-affected relatives, expanded alleles were observed exclusively in heterozygous state (13.04% individuals). In the FECD group, they were also predominantly heterozygous (60.65% subjects), although a small proportion of individuals were homozygous for the expanded allele (4.92% patients) (Table 2). The difference length distribution of *TCF4* TNR between control and FECD group is presented in Supplementary Fig. 1.

Within the FECD cohort, males showed a tendency towards a higher frequency of expanded alleles compared to females, consistent with previous reports. However, this difference did not reach statistical significance, likely due to the limited number of FECD males in the study (N = 12) in comparison to females (N = 49) (Table 2).

With the aim of evaluating co-segregation of the *TCF4* TNR expansion and FECD, further relatives were recruited for 11 families in order to have a minimum of three individuals per family in which at least two of them had FECD. To assess the role of the expanded allele in developing FECD, we evaluated the co-segregation between the presence of at least one expanded *TCF4* allele and disease status. Complete co-segregation was observed in 6 of the 11 analysed families (Supplementary Fig. 2). In the remaining 5, affected individuals without the expansion and one healthy family member carrying the expansion were identified supporting incomplete penetrance and genetic heterogeneity.

Potential use of rs613872 and rs784257 to predict the presence of TCF4 expanded alleles.

Given the observed co-occurrence of the risk variants of the rs613872 and rs784257 SNPs (Fig. 1A) and, as illustrated in Fig. 1B, the close genomic proximity between rs613872, rs784257 and the TNR expansion in the *TCF4* gene, we further investigated the presence of potential linkage disequilibrium, referring to the non-random co-inheritance of genetic variants at a frequency higher than expected, within these markers. In both groups, the observed frequency of individuals carrying all three variants exceeded that expected under independent segregation (0.130 vs 0.012 in unaffected individuals and 0.590 vs 0.311 in FECD), supporting linkage disequilibrium (Fig. 1C).

Based on these observations, we evaluated whether the presence of risk allele at rs613872 and rs784257 could predict the presence of an expanded *TCF4* allele in our cohort of 84 individuals (61 primary FECD patients and 23 non-affected relatives). We assessed different predictive models, including the presence of the rs613872 risk allele alone, rs784257 alone, both risk alleles, or either one. For each model, we calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Rs613872 alone showed the best performance, with 97.67% sensitivity, 80.49% specificity, 84.00% PPV and 97.06% NPV. Rs784257 showed lower sensitivity, while combining both SNPs did not improve performance (Fig. 1D) (Fig. 1D).

We next applied rs613872 based prediction model of *TCF4* expansion status to the 46 individuals included in the family based *TCF4* expansion FECD co-segregation study (described in the Materials and

Methods section). This cohort included 11 families, including 1 unaffected and 14 affected relatives not analysed previously. In this cohort, rs613872 achieved a sensitivity of 96.97%, specificity of 84.62%, PPV of 94.12%, and NPV of 91.67% yielding results highly consistent with those obtained in the initial cohort. These findings further support rs613872 as a robust predictor of expanded *TCF4* alleles.

Clinical characterisation of FECD families carrying TCF4 expanded alleles.

To investigate potential clinical differences between FECD patients with and without expanded *TCF4* alleles, we first compared affected individuals with non-affected controls. To minimise the influence of age-related changes, only individuals aged 50–69 years were included. Clinical data from both eyes were analysed. Due to age limitations, the cohort comprised 19 non-affected individuals and 34 FECD patients, of whom 10 had undergone corneal transplantation (Table 3). Data from transplanted eyes were excluded, and when available, preoperative measurements were used instead. As expected, FECD patients showed a near-significant reduction in visual acuity, a significant decrease in CEC density, increased central CCT, and reduced BAD values compared to non-affected individuals (Supplementary Fig. 4).

Comparison of clinical parameters between FECD patients with (N = 22) and without (N = 12) *TCF4* expanded alleles revealed no significant differences between the two groups (Fig. 2A). However, a higher proportion of patients without *TCF4* expansion had undergone corneal transplantation (41.67%) than those carrying the expansion (27.27%) (Table 3).

To reduce genetic and clinical heterogeneity that could mask the effect of the TNR expansion, we performed a family-based analysis focusing on four pedigrees in which both expanded and non-expanded affected individuals coexisted within the same generation. The expanded group included 8 individuals and the non-expanded group 4. All the individuals included in this study are indicated with an asterisk in Supplementary Fig. 3. The results showed that none of the individuals in the non-expanded group had undergone corneal transplantation, whereas 2 out of 8 individuals in the expanded group had received a transplant (family 9, II.6; family 11, II.4) and this group also exhibited a higher frequency of cataracts (Table 3). Notably, the expanded group showed significantly worse visual acuity and increased CCT compared to the non-expanded group (Fig. 2B). These findings support an association between *TCF4* expansion and increased disease severity.

Discussion

SNPs and TNR expansion in TCF4 are not independent genetic risk factors for FECD.

Previous studies have identified several *TCF4* SNPs including rs11659764, rs784257, rs613872, rs17089887 and rs17595731(1, 2, 30, 32, 35, 37, 49–54) as well as TNR expansion in *TCF4* (1–5, 30–38), as genetic variants associated with the development of FECD. Among these, the rs613872 risk allele and expanded *TCF4* TNR length have been the most extensively studied across multiple cohorts, consistently

showing a strong association with disease susceptibility. However, this relationship had not been previously evaluated in a Spanish cohort.

In the present study, we initially analysed 61 unrelated individuals with FECD and 23 unaffected relatives and observed a higher frequency of the risk alleles for rs613872 and rs784257, as well as expanded *TCF4* TNR alleles, in affected individuals (Tables 1 and 3).

Both SNPs and the TNR expansion are located in non-coding regions and in close proximity (Fig. 1B). Although these SNPs have not been directly linked to functional consequences, several mechanisms have been proposed to explain how TNR expansion may disrupt *TCF4* function including altered isoform-specific TCF4 expression, toxic RNA foci, sequestration of RNA-binding proteins, missplicing, repeat-associated non-AUG translation and somatic instability(2, 31, 33, 39, 40, 42–48).

To investigate whether SNPs and TNR expansion act as independent genetic risk factors, we analysed the co-occurrence of these variants. In both affected and non-affected groups, the practical frequencies of individuals carrying all three genetic variants simultaneously exceeded the theoretical frequencies expected under independent segregation (Fig. 1C). Their genomic proximity and non-random co-ocurance are consistent with linkage disequilibrium, likely due to a shared evolutionary origin and reduced recombination in this region, resulting in their co-inheritance at rates higher than expected by chance.

Taken together, these findings support the hypothesis that *TCF4* TNR expansion constitute a pathogenic mechanism underlying FECD. In contrast, the associated SNPs are likely co-inherited through linkage disequilibrium and therefore may not represent independent genetic risk factors per se.

rs613872 can be used as a predictor tool for the presence of TCF4 TNR expansion.

TCF4 TNR expansion is commonly characterised using STR and TP-PCR which are technically demanding since require multiple specialised primers, careful optimisation of PCR conditions, high-quality DNA, and access to capillary electrophoresis platforms, with results that can be complex to interpret(3, 32, 40, 55).

In contrast, SNP genotyping by Sanger sequencing is technically less complex and widely accessible to most laboratories. Our results show that rs613872 showed strong predictive performance for *TCF4* expansion in our cohort (Fig. 1D). and may therefore serve as a simple surrogate marker where STR and TP-PCR are not readily available. Notably, this should not be used as a predictive tool for the development of FECD, as further deliberated in the following section of the Discussion.

TCF4 TNR expansion is an important genetic risk factor for FECD development but does not appear to be the primary causal factor.

Our results show a strong association between the presence of expanded *TCF4* alleles and FECD. Approximately two-thirds of FECD patients carried one or two expanded alleles, whereas only one-tenth

of non-affected individuals presented a single expanded allele (Table 2).

However, *TCF4* expansion is more frequent in males than in females (Table 2). This contrasts with the epidemiology of FECD, which is more prevalent in females, suggesting that *TCF4* expansion alone does not fully explain disease susceptibility.

FECD affects approximately 4–7% of the adult population worldwide. Nevertheless, there is marked familial variability: some families show no affected individuals, whereas others present multiple affected members. This supports a strong genetic component, with shared risk factors likely contributing to disease development within the individuals of the same families. To further investigate this, we evaluated the co-segregation of *TCF4* TNR expansion and FECD development in 11 FECD families. Complete co-segregation was observed in only about half of the families, while affected individuals without expansion and an unaffected expansion carrier were identified (Supplementary Figs. 2 and 3). In this regard, incomplete penetrance between *TCF4* TNR expansion and FECD was already reported in some published studies(34, 36, 55).

Taken together that- (i) one-third of affected individuals lacked expanded alleles, (ii) the presence of expansion in 10% of healthy relatives, (iii) incomplete co-segregation in half of the families, and (iv) the discordance between the sex distribution of expansion and disease prevalence- we hypothesise that *TCF4* TNR expansion, while an important genetic risk factor, is not sufficient or deterministic for disease development.

FECD is characterised by a high degree of clinical heterogeneity, with features that are commonly observed but not consistently present across all patients. There is also notable variability in disease severity, including differences in the need for corneal transplantation and in the age at disease onset with early-onset phenotypes linked to *COL8A2* variants(18–21). Moreover, histological classification of DMEK samples from FECD patients has identified five distinct lesion groups, some associated with the presence or absence of *TCF4* TNR expansion(56). This clinical variability may reflect underlying genetic heterogeneity.

Taken into consideration this background, we explored whether the *TCF4* expansion is more closely associated with disease severity rather than disease onset. We evaluated clinical characteristics within our cohort. In our dataset, which included unaffected and affected individuals aged 50–69 years to minimise variability related to age, we observed clear differences between groups including reduced visual acuity, decreased endothelial cell density, increased CCT, and reduced BAD values (Supplementary Fig. 4). However, these differences were not observed when comparing affected individuals with and without *TCF4* TNR expansion, likely due to the high variability and limited sample size (Fig. 2A).

To reduce heterogeneity, we performed stratified analysis focusing on four families, comparing individuals from the same generation within the same family with and without *TCF4* TNR expansion. Although this approach reduced sample size, we observed worse visual acuity, increased CCT and a higher frequency of cataracts and corneal transplantation in individuals carrying the expanded allele

(Fig. 2B and Table 3). While these findings would benefit from validation in a larger number of families, they support a potential association between *TCF4* TNR expansion and increased disease severity rather than disease initiation. This interpretation is consistent with several previous studies, although the exact role of the expansion in modulating FECD phenotype remains a topic of ongoing debate(1, 3, 4, 34, 41).

Conclusions

Taken together, our results indicate that *TCF4* TNR expansion represents the main genetic risk factor associated with FECD, while SNPs within *TCF4* are not independent risk determinants but are likely co-inherited due to linkage disequilibrium. We demonstrate that rs613872 is a strong surrogate marker for the presence of *TCF4* expansion and may serve as a simple and accessible predictive tool for expansion screening. Clinically, *TCF4* expansion is associated with increased disease severity rather than disease onset, although it is neither necessary nor sufficient for disease development, as evidenced by no co-segregation within half of the families. Overall, these findings support a model in which *TCF4* expansion contributes significantly to FECD susceptibility and severity, but additional genetic factors, including variants affecting key proteins involved in corneal endothelial function, as well as environmental influences, are likely required for full disease manifestation.

Declarations

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Conflict of Interest

The authors declare that they have no financial conflict of interest related to this study.

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Authors contributions Statement

PEF contributed to the investigation, methodology, formal analysis, data curation, visualisation, project administration, and preparation of the original manuscript draft, and participated in reviewing and editing the manuscript. MJ contributed to the investigation, methodology, and data curation. LG contributed to the conceptualisation and methodology of the study, provided resources, participated in project administration, and acquired funding. SB and SSB contributed to the investigation and data curation. OG and CMC contributed to the investigation and provided clinical resources. JLG contributed to the conceptualisation and investigation of the study, provided resources, and supervised the work. EP contributed to the conceptualisation and methodology of the study, provided resources, supervised and administered the project, and participated in reviewing and editing the manuscript. All authors reviewed and approved the final version of the manuscript.

Supplementary information is available at EYE's website.

Competing Interests

This research was funded through the DISENFUCHS Project, a study of genotype–phenotype correlations in patients with Fuchs endothelial corneal dystrophy, by MINIOPTERUS INVESTIGACIONES, A.I.E. and its participating entities, in collaboration with INVENTIUM.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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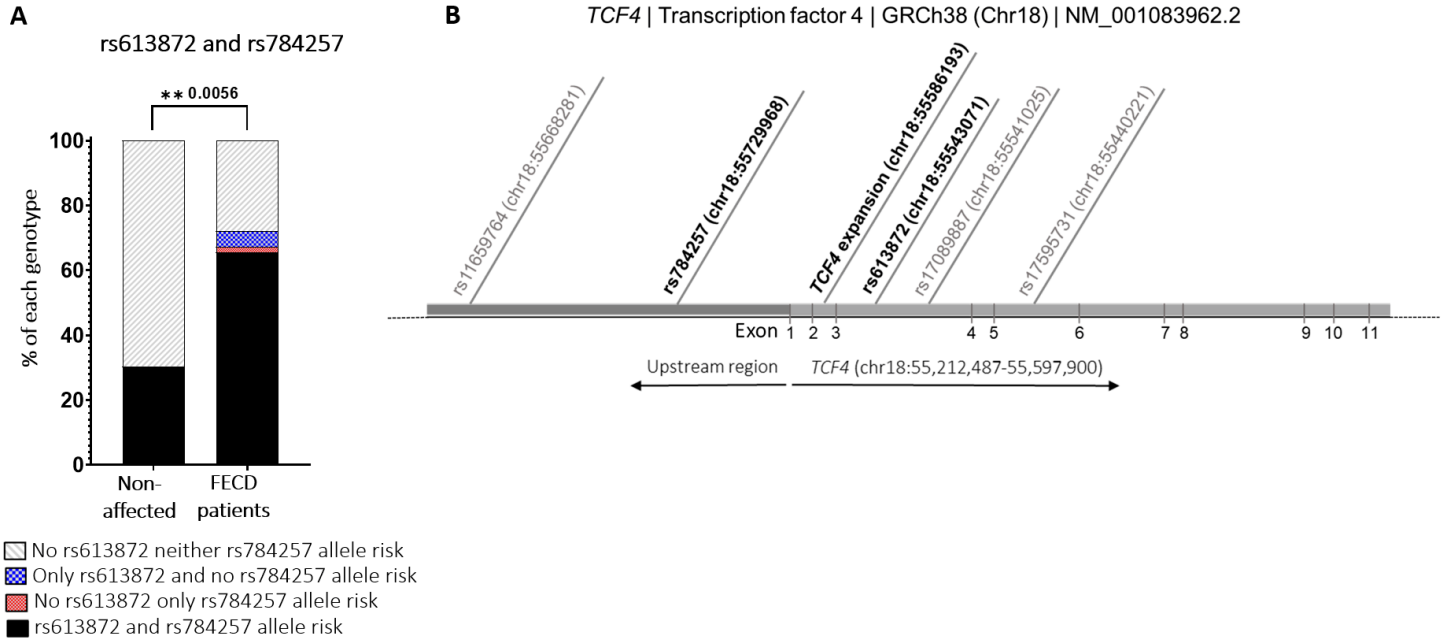
Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures



C

	rs613872	rs784257	TNR *TCF4*	rs613872 + rs784257 + TNR *TCF4* expansion	
	Practical frequency	Practical frequency	Practical frequency	Theoretical frequency	Practical frequency
Non-affected (N=23)	0.304	0.304	0.130	0.012	0.130
FECD (N=61)	0.705	0.672	0.656	0.311	0.590

D

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
	$SE = \frac{TP}{TP + FN}$	$SP = \frac{TN}{TN + FP}$	$\text{PPV} = \frac{TP}{TP + FP}$	$\text{NPV} = \frac{TN}{TN + FN}$
rs613872	97.67%	80.49%	84.00%	97.06%
rs784257	90.48%	80.95%	82.61%	89.47%
rs613872 plus rs784257	90.70%	78.05%	81.25%	88.89%
rs613872 or rs784257	97.76%	78.05%	82.35%	96.97%

Figure 1

Linkage disequilibrium analysis for rs613872, rs784257 and TCF4 TNR expansion. A) Distribution of individuals carrying the risk alleles of rs613872 and rs784257, either individually or in combination, in non-affected controls (N = 23) and FECD patients (N = 61). Heterozygous and homozygous carriers are not distinguished. Differences in distribution between groups were assessed using the chi-square (χ^2) test. P-values are reported, with statistical significance indicated as ** for p-value < 0.01. B) Genomic localisation of rs613872, rs784257 and *TCF4* TNR expansion on chromosome 18. Exons are annotated.

C) Observed frequencies were calculated in both groups (N = 23 non-affected individuals and N = 61 FECD patients) for each genetic variant, as well as for the co-occurrence of rs613872, rs784257, and *TCF4*TNR expansion. The observed frequency of this combined genotype (rs613872 + rs784257 + *TCF4* TNR) was compared with the expected frequency, which was estimated under the assumption of independence between events as the product of the individual event probabilities, in both non-affected individuals and FECD patients. D) Evaluation of the predictive performance of each SNP combination for identifying expanded *TCF4*alleles in our cohort of 84 individuals (61 FECD patients and 23 non-affected relatives), including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

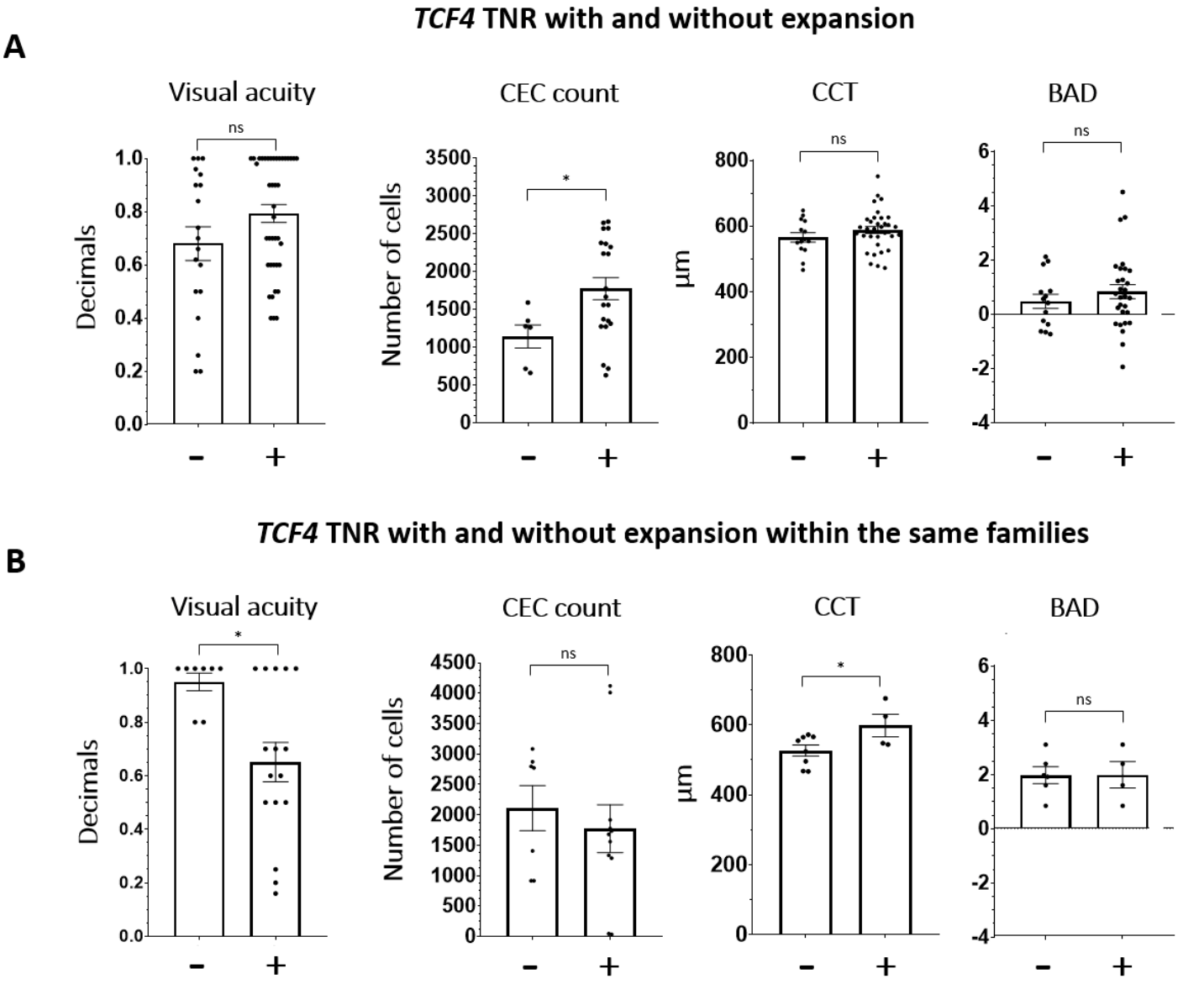


Figure 2

Clinical characterisation of FECD patients with and without *TCF4* expanded alleles. A) Visual acuity, CEC count, CCT and BAD measured in FECD patients with *TCF4* TNR expansion (N=22) and without expansion (N=12) aged 50-69 years. Clinical data from both eyes were analysed. Data from transplanted eyes were excluded (6 individuals in the FECD with expansion group and 5 individuals in the FECD without expansion group) and preoperative measures were used when available. All data are presented as mean ±SEM. **B)** Visual acuity, CEC count, CCT and BAD measured in FECD patients with *TCF4* TNR expansion (N=8) and without expansion (N=4) coexisting within the same generation of the same families. Clinical data from both eyes were analysed. Data from transplanted eyes were excluded (2 individuals in the FECD with expansion group and none individuals in the FECD without expansion group) and preoperative measures were used when available. CEC values equal to 0 were excluded, as they were considered unreliable measurements. All data are presented as mean ±SEM. CEC: corneal endothelial cells; CCT: central corneal thickness; BAD: Belín-Ambrosio Enhanced Ectasia Display Total Deviation Value. Significant differences between groups were assessed using an unpaired T-test. P-values are reported, with statistical significance indicated as * for $p < 0.05$. - : without expansion, + : with expansion.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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